RESPONSES OF PLANTS TO RHENIUM

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Received April 22, 1953

Preliminary studies of the effects of rhenium salts on plants were initiated by the junior author at Harvard University during 1946 and 1947 under the direction of Professor K. V. Thimann. The interest in this metal was stimulated by his results in an earlier project testing physiological effects of various unusual elements for which such data were not as yet available. In this project, which included elements such as tellurium, gallium, gold, etc., the effects of rhenium were found to be unique. The symptoms produced were striking, and they were produced at very low concentrations. The information obtained was made available to the senior author who in turn supervised a series of student projects bearing on this problem as part of his courses in plant physiology. Confirmation of the original observations was obtained, and the investigations were expanded to probe the mechanism involved.

The element rhenium (Re), atomic number 75 and atomic weight 186.31, is classified with manganese, technetium, and neptunium in group VII-A of the periodic system of the elements. As a metal, rhenium is interesting because of its high melting point (3440 ± 40° C). The element is unique in its reported ability to achieve all possible valence states from -1 to $+7$. Metallic rhenium is readily available at a cost of about $3.60 per gm.

Solutions were prepared by dissolving a known weight of metallic rhenium in concentrated HNO_3, adjusting to the desired pH (pH 6 in recent work) with NaOH, and diluting to desired strength with distilled water. At higher pH values precipitation occurs. It was also found that solutions must be reasonably fresh to give consistent results since a continual, gradual crystallizing out occurs even in the lower pH range.

Several different procedures for introducing rhenium into the plants were used, all of which proved effective. These included (1) a foliar spray with a nasal atomizer, (2) subepidermal injection with a hypodermic syringe, (3) direct addition to the culture solution surrounding the roots, and (4) direct addition to the soil. Solutions containing from 0.1 to 200 mg. Re/l. have been employed.

The nature of the responses varied among different plants. In a series of experiments in which rhenium solutions were applied to sand cultures, it was found that concentrations of 1 mg./l. or less produced no observable effects in all experiments. Concentrations between 5 and 50 mg./l. (2.68×10^{-5} to 2.68×10^{-4} M) resulted in darkening of the green coloration and in increasingly severe morphological modifications. Concentrations above 50 mg./l. generally proved lethal. In the monocots corn and wheat, the coleoptiles of the seedlings appeared to toughen and thicken so that the stem did not break through in the normal manner. Twisting and malformation of seedlings together with apparent fusion of the sheaths of the young leaves to the stem resulted. The general appearance in advanced cases was one of hypertrophic expansion of growing portions. If treated for a time just sufficient for development of symptoms, then returned to normal conditions, the plants recovered. The treatment and recovery could be repeated several times. Young dicotyledonous plants, such as tomato and pea, exhibited the same increased darkening of the green coloration together with progressively increasing scattering of small brownish spots which were more abundant toward the margins of the blades. In the higher and more toxic concentrations, the leaflets cupped under, the leaves curved backward, and necrosis developed in the young leaves and terminal leaflets of mature leaves and progressed downward through the plant. Symptoms developed in tomato plants within two days, while death occurred within five days, in tomato plants exposed to 100 mg./l. spray. Plants which have responded to spray applications of 50 mg. Re/l. include tomato, bean, pea, corn, wheat, oat, geranium, and coleus.

Exploratory experiments were undertaken to investigate the mechanism of toxicity. Among these were the similarity between rhenium toxicity and those of manganese and phosphorus, the effects of the element on cell division, and its effects on respiration. Young soil-grown tomato plants were transferred to mineral solution culture and allowed to recover for a period of a week. At the end of this period, manganese chloride was added to the solutions so as to give a series of experimental plants exposed to concentrations of manganese including 0.0, 1.0, 10, 100, 500, 1000, and 2500 mg./l. Simultaneously, comparable plants were similarly treated with 50 mg. of rhenium per liter. At none of the concentrations of manganese were the symptoms similar to or nearly so marked as those developed in 50 mg. Re/l. The threshold of toxicity was at a lower concentration in the case of rhenium ($\pm 2.68 \times 10^{-4}$ M) than for manganese ($\pm 1.82 \times 10^{-2}$ M). Experiments probing the toxic symptoms of phosphorus have been inconclusive because of the difficulty in maintaining toxic concentrations of phosphates in these calcium-containing solutions. Deficiency symptoms of phosphorus were not found to be identical to those produced at a toxic concentration of rhenium. An idea of of the dosage required to produce symptoms was gained by a study of sand cultures of tomato. Plants were watered daily and in the watering received a supplement of 25 ml. of complete nutrient solution. Experiments were begun on plants 7 to 8 inches tall by supplying them with 1.25 to 2.50 mg. of rhenium per day in addition to the usual nutrient supplement. On the 7th day, after a total dosage of 11.25 mg./plant, symptoms began to develop.

The effects of rhenium on cell division were studied by using cultures of the alga *Coelastrum* (Yale strain) grown in Chu 10 medium (1) containing different concentrations of rhenium. Growth was followed spectrophotometrically over a three-week period. Concentrations of 1 mg./l. or less showed no reduction in growth when compared to controls. Between 5 and 50 mg./l. an increasing reduction in optical density was found. Nearly total inhibition of growth occurred at 80 mg./l. These values correspond quite closely with concentrations found effective in the angiosperms previously tested. This effect of rhenium on the rate of cell division in *Coelastrum* stimulated a cytological study. Well-developed onion roots were exposed to 50 mg./l. solutions of rhenium for 24-, 48-, and 72-hour periods, after which the root tips were fixed in Carnoy's fixative (3 : 1) and smeared in acetocarmine. No irregularities in chromosome morphology or mitotic figures were detected when examined under magnifications up to 1250 ×.

The effect of rhenium on respiration was studied using *Chlorella* (Yale strain) grown in Maerten's solution and employing the Warburg technique at 25° C. The formulation for Maerten's solution was provided by Dr. Ralph Lewis and Dr. Joyce Lewin, now of Halifax, Nova Scotia. In the run, oxygen uptake was followed for a period of an hour prior to addition of rhenium from the sidearm, then followed for an additional hour. Concentrations of 1.0 and 80 mg. Re/l. produced no detectable changes in rate of gas exchange. In order to check these results, and to eliminate possible interference by constituents of the culture solutions previously used, a comparable experiment was run using sections of potato tuber. These sections from a fresh potato were 10 mm. in diameter and 75 μ thick, and were washed in running tap water for 24 hours prior to experimentation. Concentrations including 1, 50, and 100 mg. Re/l. were employed. No important differences in oxygen uptake were detected during the $1\frac{1}{2}$ hours subsequent to mixing, although one manometer registered a marked decrease in oxygen uptake during the run. Since in this earlier experiment an indication of possible decline in rate of respiration was noted, a run was made to determine changes in the rate over longer periods of time. A concentration of 100 mg. Re/l. was used in the experimental flasks, and the time of exposure of potato tuber slices to rhenium was increased to five hours. Whereas no effect was detected during the first hour, consistent with the previous data, during the longer five-hour run a gradual decline in respiration was observed. After five hours, the oxygen uptake had dropped from 0.07 μl. O_2/mg. dry weight × hr. to 0.04 μl. O_2/mg. dry weight × hr., an inhibition of approximately 43% in five hours. The control exhibited no decline in rate of oxygen uptake during this period. From these studies, rhenium appears to have a pronounced but delayed depressing effect on the rate of respiration.

The basis of the marked darkening of green coloration was studied by extracting normal and symptom-exhibiting leaves with 80% methanol. The absorption spectra obtained with a Coleman Universal Spectrophotometer were practically identical. Thus, the color change was apparently not the result of alterations in the nature or concentration of the chloroplast pigments. Extraction for non-chloroplast pigments has not as yet been undertaken.

Summary

Summarizing the above information, marked response by plants to rhenium is noted. The symptoms are severe and develop fairly rapidly. Concentrations from 5 to 50 mg./l. produced increasingly intense symptoms, while those in excess of 50 mg./l. are generally lethal. The total dosage required to produce symptoms in tomato was approximately 11 mg. Darkening of the green coloration is apparently not due to changes in chloroplast pigments. The symptoms are not the same as those of manganese toxicity, and apparently differ from those of phosphorus deficiency. Cell division is reduced, but no obvious modifications in chromosome morphology or mitotic figures were detected. Respiration is inhibited, the inhibition being gradual and detected only after several hours of exposure to toxic concentrations. The mechanism of operation is not yet known, but the effects of rhenium on rate of cell division and rate of respiration suggest avenues of approach to the problem.

The authors gratefully acknowledge the assistance of Messrs. H. Rogelio Cuellar, Charles Dame, Clinton K. Davis, Jr., Herbert T. DeRigo, John A. Jagschitz, Charles H. Lamoureux, Charles Mason, and Robert E. Partyka. For the cytological work, the authors are especially indebted to Prof. Robert Lepper, Jr., Head of the Botany Department.

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